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Association between Paraoxonase -1 Gene Promoter T (-107) C Polymorphism and Coronary Artery Disease

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Abstract

Background: Paraoxonase-1(PON1), a high-density lipoprotein (HDL) associated enzyme, is believed to contribute in the pathogenesis of coronary artery disease (CAD). The aim of this study was to evaluate the association of PON1 promoter C (-107)T polymorphism with the extent of coronary artery stenosis in Iranian patients.

Methods: The RFLP analysis for determination of the C(-107)T genotype distribution and measurement of serum PON1 activities (Paraoxonase and Arylesterase) were performed in 99 patients. They were undergone coronary angiography to determine the number of stenotic vessels and classified into three groups: single vessel disease (SVD), two vessels disease (2VD) and three vessels disease (3VD).

Results: The C(-107)T polymorphism was significantly associated with serum arylesterase activity but not with paraoxonase activity. The CC and TT genotypes distributed inversely in SVD as compared with 3VD group. Moreover, the CC high activity genotype frequency decreased with increase of stenotic vessels in patients.

Conclusion: The reduced arylesterase activity as a function from the weak promoter activity increases the stenosis severity, so that, we assume it is one of the progressive factors of atherosclerotic process in stenotic vessels.

Keywords: CAD, C (-107) T polymorphism, Stenosis severity, Iran

Introduction

Paraoxonase-1(PON1), a 45-kDa glycoprotein (1), is a calcium dependent esterase that hydrolyzes thiolactones (2), arylesters such as phenyl acetate and organophosphates such as paraoxon, sarin, diazoxon and soman (3). The role of serum PON1 as one high-density lipoprotein (HDL) associated enzyme have studied in several diseases (4-7). Coronary artery disease (CAD) is thought to begin with inflammatory reactions at the endothelial level, and oxidized LDL is believed to be center to progression of atherosclerosis (8). Paraoxonase (PON1) as an antioxidative enzyme protects LDL from oxidation by the hydrolysis of lipoperoxides (9). Thus, it has been suggested that the serum PON1 activity is markedly related with CAD risk in human (10). Nu-

merous polymorphisms in the coding region and promoter of the PON1 gene affect the enzymatic activities (arylesterase and paraoxonase) (11-12). The role of coding region polymorphisms in predicting atherosclerosis remains controversial; some studies revealed a significant association between these polymorphisms and CAD risk, while others reported a lack of association (13-14). Several studies have also focused on the PON1 promoter polymorphisms and shown that the C (-107) T position within Sp1 consensus is related with CAD risk. The TT genotype associated with low arylesterase activity has been suggested as a risk factor of CAD by several investigators (15) but not by others (16).

The present study was to evaluate the serum PON1 activities and C (-107) T polymorphism to define

whether polymorphic site as an independent factor was associated with the severity of coronary artery stenosis in Iranian patients.

Materials and Methods

Ninety nine subjects with CAD were selected from September 2006 to March 2007. Subjects who underwent coronary angiography in Tehran Rajaee Cardiovascular Center for detection of the extent of stenosis in coronary artery vessels were recruited during the course of a study of genetic risk factors for CAD according to a designed protocol. Patients were included at least a 70% stenosis in a major epicardial artery and subdivided into three groups: single vessel disease (SVD), two vessels disease (2VD) and three vessels disease (3VD). Participants with hepatic or renal disease, cardiomyopathy, congestive heart failure and acute myocardial infarction within the last three months were excluded from the study. Medical history, drug intake and demographic data of the study population were collected on a questionnaire. Blood pressure, weight and height were also recorded.

Blood was drawn from all subjects after an overnight fast. Serum was separated immediately and stored at -20 °C. Genomic DNA was extracted from whole blood by the method of Miller et al. (17) and stored at -20 °C.

Plasma total cholesterol and triglycerides were measured by routine enzymatic methods. HDL-cholesterol was determined after precipitation of the apoB-containing lipoproteins. LDL-cholesterol was calculated using Friedewald formula (18).

Paraoxonase-1 activities toward phenylacetate (Sigma Co, USA) and paraoxon ethyl (Sigma Co, USA) were determined spectrophotometrically at 270 nm and 412 nm, respectively. One unit of arylesterase activity was equal to one μ mol of phenylacetate hydrolyzed per ml per minute. In addition, one unit of paraoxonase activity provided one nmol p-nitrophenol per ml per minute (19).

The C(-107)T genotype frequency was determined with the RFLP analysis method as described by Brophy et al. (20).

The data were analyzed using SPSS 13.0 and presented as mean \pm SD. The PON1 arylesterase activity differences between genotypes were also evaluated by analysis of variance (ANOVA) followed by post hoc testing with Turkey's test. Categorical variables were compared between groups by the χ^2 test. $P < 0.05$ were considered to be significant for statistical tests.

Results

Ninety nine subjects (73 men and 26 women) were studied (Table 1). Patients were taking aspirin and lipid- and blood pressure-lowering medications. No significant differences in mean age, BMI, blood pressure and lipid profiles were found between the patient groups.

The mean of PON1 activity toward paraoxon ethyl was insignificantly different among the genotypes, although, the TT genotype had a lower activity than did the other genotypes (Table 2). In contrast, the increase of PON1 activity toward phenyl acetate was significantly associated with the number of -107 C allele. We observed a reverse and significant association between the increase of -107T allele and decreased arylesterase activity in the SVD and 3VD groups.

The results were significantly showed reverse association between the number of stenotic vessels and PON1 arylesterase ($P = 0.002$) and paraoxonase ($P = 0.038$) activities as evaluated by ANOVA (Fig. 1). The arylesterase and paraoxonase activities decreased 22% and 20%, respectively in 3VD as compared with SVD.

The genotype distribution of C (-107)T polymorphism are reported in Fig. 2. The CC frequency as a high activity genotype was higher in the SVD as compared with 2VD and 3VD, while the frequency of low activity genotype (TT) had direct association with the stenosis severity in coronary arteries.

Table 1: Characteristics of patients with coronary artery disease

Parameter	Patients (n= 99)			
	SVD	2VD	3VD	All
Age (yr)	59.34±9.42	55.12±9.06	58.34±9.38	58.31±9.21
Sex (Men/Women)	(19/16)	(12/6)	(42/4)	(73/26)
BMI (kg/m ²)	26.81±3.32	25.17±6.4	25.8±5.06	26.23±4.37
SBP(mmHg)	123.8±1.6	126.0±0.9	121.5±1.14	123.3±1.3
DBP(mmHg)	77.1±0.4	80.0±0.7	78.4±0.3	78.0±0.4
Cholesterol(mg/dl)	173.14±37.04	166.44±39.07	170.17±36.28	170.51±36.00
Triglycerides(mg/dl)	139.40±72.83	138.63±49.82	151.09±58.30	146.12±64.23
HDL-C(mg/dl)	41.49±9.81	36.44±5.60	39.07±9.42	39.44±9.16
LDL-C(mg/dl)	94.44±25.97	90.87±21.56	93.45±23.07	93.24±22.89

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index

Table 2: The C(-107)T genotype frequency and PON1 paraoxonase and arylesterase activities in subjects

Enzyme activity (U/mL)	Genotype (Frequency)	Patients (Mean±SD)			
		SVD(35)	2VD(16)	3VD(46)	All (99)
Paraoxonase					
	CC(0.27)	255.91±94.41	264.40±97.55	254.77±89.26	258.01±90.22
	CT (0.48)	290.71±113.33	270.43±52.70	215.59±73.31	249.6±95.13
	TT(0.25)	225.10±76.81	198.91±45.71	211.84±69.07	210.76±63.35
Arylesterase	P-Value	NS	NS	NS	NS
	CC (0.27)	122.53±28.04	109.58±15.62	106.28±13.13	109.79±27.40
	CT (0.48)	105.62±24.39	83.58±15.62	87.03±23.80	96.24±25.87
	TT (0.25)	65.76±19.44	82.34±21.00	76.13±28.10	76.24±25.01
	P-Value	<0.01	NS	<0.05	<0.01

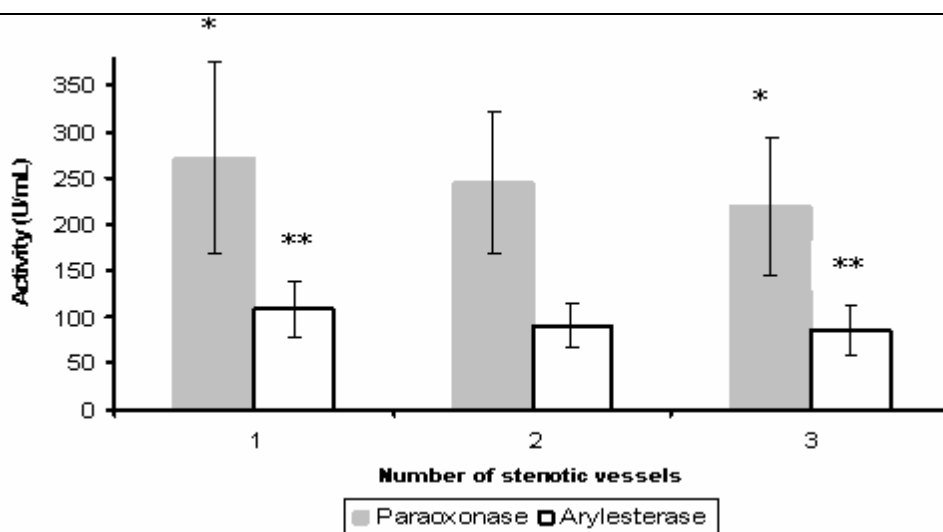


Fig. 1: Relationship between PON1 arylesterase and paraoxonase activities and the number of stenotic vessels. *, **, $P < 0.05$ between SVD and 3VD groups

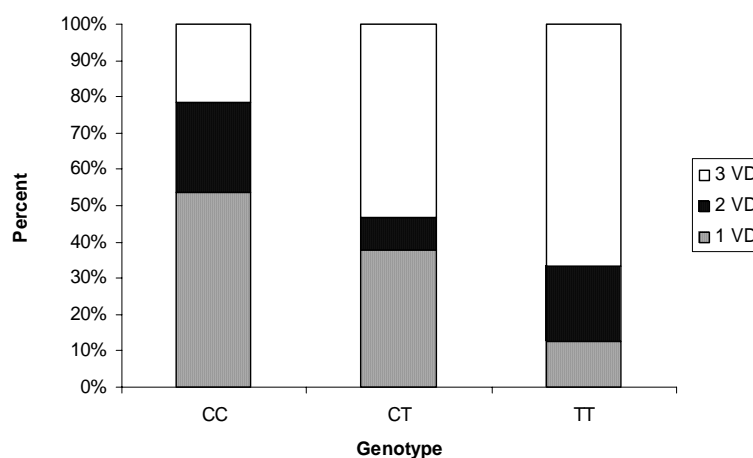


Fig. 2: The C(-107)T genotype distribution among CAD patients

Discussion

The present study confirmed association between the PON1 gene promoter C(-107)T polymorphism and extent of coronary artery stenosis in Iranian patients with CAD. Since, no significant differences were observed between the characteristics of patient groups, thus, the results were brought about the changes of C(-107)T polymorphism and the PON1 activities.

The role of some common polymorphisms such as -107, -162, -824 and -907 within the 5' region of the PON1 gene have studied with CAD risk by several investigators (21-22). In vitro expression studies have been demonstrated that the -107 position occurs within a consensus sequence for the transcriptional factor Sp1 and is related with the PON1 gene expression (23). Moreover, the serum arylesterase activity has indicated as a function from the PON1 promoter activity, while the paraoxonase activity has involved with the Q192R polymorphism within the PON1 coding region (24).

In agreement with these studies, our data showed a significant association between the number of C-107 allele and the serum arylesterase activity, so that the inverse patterns from distribution of the TT and CC genotypes found among the patient groups. The results showed no association between paraoxonase activity and C(-107)T polymorphism. It was logical due to the demon-

strated association between the PON1 promoter polymorphism with arylesterase activity but not with paraoxonase activity (24).

In our patients, the arylesterase and paraoxonase activities decreased in agreement with the stenosis severity due to the weak promoter activity associated with the -107T allele. These results hypothesize that the PON1 activity changes antioxidative capacity, this, in turn, affects the extent of stenosis in coronary arteries.

In conclusion, the C(-107)T polymorphism is associated with the PON1 arylesterase activity but not with paraoxonase activity. Furthermore, the reduced arylesterase activity as a function from the weak promoter activity increases the severity of CAD, so that, we assume it is one of the progressive factors of atherosclerotic process in stenotic vessels.

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The authors declare that they have no Conflict of Interests.

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